Halomonas radicis sp. nov. isolated from *Arthrocnemum macrostachyum* growing in the Odiel marshes (Spain) and emended descriptions of *Halomonas xinjiangensis* and *Halomonas zincidurans*

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain EAR18^T is KU320882. The GenBank/EMBL/DDBJ accession numbers for *gyrB*, *secA*, and *atpA* gene sequences are MH925812, MH925813, and MH925814, respectively. The GenBank/EMBL/DDBJ accession number for the draft genome sequence of strain EAR18^T is CAAHFN01.

Abstract

Strain EAR18^T was isolated as an endophyte from the roots of a halophyte plant, *Arthrocnemum macrostachyum,* growing in the Odiel marshes (Huelva, Spain). Strain EAR18^T was Gram staining-negative, motile, non-spore forming, and aerobic rods. It optimally grew on TSA medium supplemented with 2.5 % NaCl (w/v), at pH 7 and 30 ºC for 48 h. It tolerated NaCl from 0 % to 25 % (w/v). It presented Q9 as the major quinone and $C_{19:0}$ cyclo ω8*c*, Summed feature 8 ($C_{18:1}$ ω7*c* and/or $C_{18:1}$ ω6*c*), and $C_{16:0}$ as the predominant fatty acids. The polar lipids profile consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, and four unindentified phospholipids. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain EAR18^T formed a well-supported clade with species *Halomonas zincidurans* B6^T and *Halomonas xinjiangensis* TRM 0175^T (similarities of 98.3 % and 96.1 % respectively). Furthermore, dDDH resulted in values of 20.4 % with *H. xinjiangensis* TRM 0175^T and 35.50 % with *H. zincidurans* B6^T, and ANIb/ANIm results in values of 73.8 %/84.2 % with *H. xinjiangensis* TRM 0175^T and 86.8 %/89.4 % with *H. zincidurans* B6^T. Based on phylogeny and differential phenotypic properties showed when compared with the closest related species, strain EAR18^T is suggested to represent a new species in the genus *Halomonas*, for which the name *Halomonas radicis* sp. nov. is proposed. The type strain is EAR18^T (=CECT 9077^T=LMG 29859^T). The whole genome was sequenced, and it has a total length of 4.6 Mbp and a G+C content of 64.9 mol%.

Keywords: endophyte, *Halomonadaceae,* Plant growth-promoting bacteria, PGPB, heavy metals.

The genus *Halomonas* is placed into the family *Halomonadaceae* [1,2,3,4], a monophyletic group belonging to the class *Gammaproteobacteria*. The genus *Halomonas* was first proposed in 1980 by Vreeland *et al*. [5] and later emended by Dobson and Franzmann [2]. At the time of writing, *Halomonas* comprises 94 species [6] being the type species *Halomonas elongata* [5]. Representatives of the genus have been mainly isolated from saline lakes [7,8], fermented seafood [9,10], saline soils/sediments [11,12,13,14,15], and marine environment [16,17], although recently they were also found associated with plant rhizosphere [18] and inhabiting plant stems [19].

Strain EAR18^T was an endophytic bacterium isolated from roots of an *Arthrocnemum macrostachyum* plant growing in the Odiel marshes (37º 13'N - 6º 57'O) (Huelva, Spain) [20]. 16S rRNA gene sequence analysis of EAR18^T already showed that this strain belongs to the genus *Halomonas* [20]. Strain EAR18^T exhibited *in vitro* tolerance to high concentrations of heavy metals such as Cd and Cu (minimum inhibitory concentration 2 mM) and *in vitro* plant growth-promoting properties in absence and presence of heavy metals [20]. Additionally, beneficial effects on seed germination, plants growth in presence of polluted sediments, and as a biostimulator of phytoaccumulation of heavy metals in *A. macrostachyum* were demonstrated [20,21].

In this study, we determined the phylogenetic position of the isolate EAR18^T following a polyphasic characterization and the recommended minimal standards indicated for the family *Halomonadaceae* [22]. Based on both phylogenetic and phenotypic results, strain EAR18^T is proposed to represent a novel species in the genus *Halomonas* with the name *Halomonas radicis* sp. nov.

Strain EAR18^T was isolated from roots of *A. macrostachyum* on Tryptic Soy Agar (TSA) plates supplemented with 0.3 M NaCl and incubated at 28ºC for 72 h as outlined by Navarro-Torre *et al.* [20]. Briefly, plant roots were disinfected and mashed with sterile saline solution (0.9 % (w/v)) in a sterile mortar. The obtained paste was directly plated on TSA plates with 0.3 M NaCl and incubated at 28 ºC for 72 h. Different isolates selected based on colony morphology were sub-cultured. Pure cultures were preserved in 15% glycerol at -80ºC.

Growth conditions were determined on TSA plates supplemented with 0.3 M NaCl at 4, 15, 20, 25, 28, 30, 32, 37, and 45 ºC and pH 5.0, 6.0, 7.0, 8.0, and 9.0 for 6 days. pH values were adjusted with citrate-phosphate buffers (0.1 M citric acid and 0.2 M dibasic

sodium phosphate) and Tris-HCl buffer (0.1M Tris (hydroxymethyl) aminomethane and 0.1 M HCl). NaCl tolerance was determined on mTGE (membrane Tryptone Glucose Extract) agar medium [23] from 0 % to 30 % (w/v) for 6 days at 28 ºC. Growth under anaerobic conditions was performed on semisolid TSA tubes containing 2.5 % NaCl (w/v) and 2 % agar (w/v) sealed with paraffin and incubated for 10 days at 28 ºC [24]. Additionally, bacterial growth was tested on marine agar (MA), and the two selective media Cetrimide agar and MacConkey agar supplemented with 2.5% NaCl (w/v) for 48h at 30 ºC.

Colony morphology was determined on TSA with 2.5 % NaCl (w/v) at 30 ºC for 48 h using a stereoscopic microscope (Olympus SZ61). A colour chart (RAL D2 Design) was used to determine the colony colour. Cell morphology was observed with an optical microscope with 100 × objective (Olympus CX41 microscope) after Gram staining [25]. Motility was tested in Tryptic Soy Broth (TSB) containing 0.3 M NaCl for 30 min at 30 ºC [20] using optical microscopy.

Oxidase activity was studied by adding 1 % tetramethyl-p-phenylenediamine reagent (Becton, Dickinson and Company, Mexico). The test was considered positive when bacterial biomass turned blue within 10-15 seconds. The presence of catalase activity was tested by adding a drop of 3 % H_2O_2 to a colony and bubbles appeared.

To determine biochemical characteristics and the presence of specific enzymes API 20NE, API 20Strep, and API ZYM galleries (bioMérieux, France) were used following the manufacturer's instructions. In addition, Biolog GEN III MicroPlates were used to determine the oxidation of carbon and nitrogen sources and the sensitivity to some inhibitory compounds. Bacterial suspensions at a final transmittance of 95 % were prepared using a viscous inoculating fluid (IF) C supplemented with 0.3 M NaCl. Microplates were then inoculated and incubated in an Omnilog device (Biolog) for 3 days at 30 ºC. Results were analysed with the opm package for R [26,27] v.1.3.72. The reference strains *Halomonas xinjiangensis* KCTC 22608^T and *Halomonas zincidurans* JCM 18472^T were tested in parallel experiments.

Respiratory quinones were extracted from freeze-dried biomass and then, separated by TLC [28] and analysed by HPLC [29]. Polar lipids were extracted from freeze-dried biomass and separated by two-dimensional TLC [28]. Different polar lipids groups were detected and identified by spraying molibdatophosphoric acid, ninhydrin, molybdenum blue, and α-napthol [30, 31]. Finally, the extraction of fatty acids was performed from bacterial biomass growing on TSA with 2.5 % NaCl (w/v) for 48 h at 28 ºC [32]

including the previously mentioned reference strains in parallel experiments. Results were analysed using the Microbial Identification System (MIDI) Sherlock Version 6.1 (TSBA40 database).

DNA extraction was performed using a G-spin™ Total DNA Extraction kit (Intron Biotechnology Ltd., Korea) according to the manufacturer's instructions. 16S rRNA gene sequence was amplified and sequenced as indicated by Navarro-Torre *et al*. [20]. 16S rRNA gene sequence was deposited in GenBank/EMBL/DDBJ data library under the accession number KU320882 and aligned with corresponding sequences of the closely related type strains retrieved by EzBioCloud server (http://www.ezbiocloud.net/eztaxon) [33]. A phylogenetic tree was constructed as previously described by Montero-Calasanz *et al*. [34] using the GGDC web server [35] available at http://ggdc.dsmz.de/. Pairwise sequence similarities were calculated using the method described by Meier-Kolthoff *et al.* [36]. Multilocus sequence analysis (MLSA) using *gyrB*, *secA* and *atpA* genes were performed as outlined by de la Haba *et al*. [37]. *gyrB*, *secA* and *atpA* genes sequences were aligned with available MLSA sequences of type strains of the genus *Halomonas* using CLUSTAL_W as implemented in MEGA7 [38]. Concatenated phylogenetic trees were inferred using Maximum Likelihood (ML), Maximum Parsimony (MP), and Neighbor-Joining (NJ) algorithms as implemented in MEGA7 [38]. Draft genome sequence of EAR18^T was obtained by MicrobesNG company (Birmingham, United Kingdom) using Illumina technology and a standard analysis pipeline. Briefly, three beads were washed with extraction buffer containing lysozyme (or lysostaphin for *Staphylococcus* sp.) and RNase A and incubated for 25 min at 37 ^oC. Proteinase K and RNase A were then added and incubated for 5 min at 65 ^oC. Genomic DNA was purified using an equal volume of SPRI (Solid Phase Reversible Immobilisation) beads and resuspended in Elution Buffer (EB). DNA was quantified in triplicate with the Quantit dsDNA HS assay in an Ependorff AF2200 plate reader. Genomic DNA libraries were prepared using Nextera XT Library Prep Kit (Illumina, San Diego, USA) following the manufacturer's protocol with the following modifications: 2 ng of DNA instead of 1 were used as input, and PCR elongation time was increased to 1 min from 30 s. DNA quantification and library preparation were carried out on a Hamilton Microlab STAR

automated liquid handling system. Pooled libraries were quantified using the Kapa Biosystems Library Quantification Kit for Illumina on a Roche light cycler 96 qPCR machine. Libraries were sequenced on the Illumina HiSeq using a 250bp paired end

protocol. Reads were adapter trimmed using Trimmomatic 0.30 with a sliding window quality cutoff of Q15 [39]. Kraken [40] was used to identify the closest available reference. The quality of data was estimated mapping the reads to this using BWA mem [41], *de novo* assembly of the reads was done using SPAdes [42] mapping the reads back to the resultant contigs, and again using BWA mem to get more quality metrics. The authenticity of genome data was confirmed by comparing the 16S rRNA gene sequence extracted from whole genome assembly and 16S rRNA gene sequence previously obtained by the conventional Sanger. The whole draft genome was deposited in GenBank/EMBL/DDBJ under the accession number CAAHFN01. For genome annotation and basics statistics, the following software and servers were used: RAST server v2.0 [43], QUAST v.4.6.3 software [44], Prokka [45], SignalP 4.1 server [46], TMHMM server v.2.0 [47], and CRISPRFinder [48]. Overall genome related indexes (OGRI) were calculated using GGCD web server [35] for the digital DNA-DNA hybridisation (dDDH) test and JSpeciesWS server [49] (http://jspecies.ribohost.com/jspeciesws) for the average nucleotide identify (ANI) test.

Strain EAR18^T was Gram staining-negative, motile, non-spore forming, and aerobic rods of 0.1 × 0.2-0.3 µm (in growth phase) (Supplementary Fig. S1). The colonies were light-orange (RAL 070 80 40), opaque, viscous, circular, and convex with smooth surface, an undulate margin, and an average size of 1 mm after incubation under optimal conditions. It is in line with other species described within the genus *Halomonas* [5,15,16,50,51], although some colour variations may occur. Strain EAR18^T grew from 15 ºC to 37 ºC, with an optimal temperature at 30 ºC. The range of pH was 6.0-9.0 having the optimum at 7.0. It was able to grow both in absence and presence of NaCl, tolerating up to 25 % NaCl (w/v; optimal NaCl concentration 2.5%) (Table 1). According to Ventosa *et al*. [52] it could be therefore considered halotolerant in agreement with other species belonging to the genus [5,11,15,18,50].

Strain EAR18^T also grew well on MacConkey agar plates in correlation to what was described in the original description of *Halomonas garicola* [9], but contrary to *Halomonas malpeensis* and *H. zincidurans* in which the absence of growth was indicated on this medium [18,50]. Similar to other species described in the genus [51], it grew well on MA. Growth on Cetrimide agar plates was nevertheless negative in agreement with other closely related species [51].

Regarding hydrolysis activities present in the strain EAR18^T, Navarro-Torre *et al*. [20] already demonstrated that it is not able to hydrolyse starch, casein, DNA or Tween 80. Additionally, it was here got that strain EAR18^T neither hydrolyses aesculin nor gelatine (Table 1). On the other hand, results obtained from API ZYM and API 20NE systems showed positive enzymatic activities for acid phosphatase, alkaline phosphatase, arginine dihydrolase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, pyrolidonyl arylamidase, urease, *α*-glucosidase, and *β*-glucosidase. Additionally, API 20NE system showed that strain EAR18^T was able to reduce nitrate to nitrite and assimilate $_D$-glucose, $_D$-mannose, and potassium gluconate. Similar results were already described for other species within the genus [9,15,16,18,19,51] with the exception of urease activity and the capability to assimilate potassium gluconate which are so far only described for the isolate EAR18^T. Finally, strain EAR18^T was catalase positive and oxidase negative in line with other species in the genus [53].

According to the Biolog system, strain EAR18^T oxidised N-acetyl-D-glucosamine, $_D$-glucose, $_D$-mannose, $_D$-fructose, $_D$-galactose, $_D$-fucose, $_L$-rhamnose, inosine, sodium lactate, $_D$-sorbitol, $_D$-mannitol, $_D$-arabitol, myo-inositol, glycerol, $_L$-alanine, $_L$-arginine, $_L$-glutamic acid, $_L$-histidine, $_L$-pyroglutamic acid, $_L$-serine, $_D$-galacturonic acid, $_L$-galactonic acid-*γ*-lactone, $_D$-gluconic acid, $_D$-glucuronic acid, mucic acid, quinic acid, $_D$-saccharic acid, *p*-hydroxy-phenylacetic acid, methyl pyruvate, $_L$-lactic acid,*α*-keto-glutaric acid, $_D$-malic acid, $_L$-malic acid, bromo-succinic acid, lithium chloride, *γ*-amino-n-butyric acid, *α*-hydroxy-butyric acid, *β*-hydroxy-butyric acid, acetic acid, and sodium formate. Moreover, it tolerated the presence of lincomycin, tetrazolium blue, and aztreonam. Similarities and differences were observed when compared with reference strains tested in parallel experiments underlining, for example, the differential reaction observed between strain EAR18^T and both reference strains for dextrin, $_D$-trehalose, pectin, $_D$-arabitol, myo-inositol, $_L$-arginine, quinic acid, and methyl pyruvate (Supplementary Fig. S2).

The predominant respiratory quinone was ubiquinone Q9 (58%), which is similar to what was outlined for the genus *Halomonas* [5], although ubiquinone Q8 was also present in significant amount (34%). Minor amounts of Q8 (<10%) were already described for other species in the genus [50,51]. The polar lipids profile of strain EAR18^T consisted of diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylethanolamine, in correlation to what was observed in this study for *H.*

zincidurans JCM 18472^T and other species of genus *Halomonas* [7,8,9], apart from four unidentified phospholipids (Supplementary Fig. S3). Differently, phosphatidylglycerol along with five unidentified phospholipids was detected in the polar lipids pattern of *H. xinjiangensis* KCTC 22608^T. Finally, the major fatty acids were $C_{19:0}$ cyclo $\omega 8c$ (29.69%), summed feature 8 ($C_{18:1}$ $\omega 7c$ and/or $C_{18:1}$ $\omega 6c$; 15.01%), and $C_{16:0}$ (19.07%). These fatty acids were also found in the closely related species studied but at different proportions in spite of fact they were cultured under the same conditions (Table 2). Inconsistencies in fatty acid profile was already observed when describing other species in the genus [51, 54] and was emphasised by Vreeland [53] when referring to the lack of specific or unique cell components that may be considered as taxonomically distinct markers for the genus *Halomonas*.

Analysis of 16S rRNA gene sequence (1322 bp) placed strain EAR18^T as a representative within the family *Halomonadaceae* showing the highest similarity with *H. zincidurans* B6^T (98.3 %), *H. xinjiangensis* TRM 0175^T (96.1 %), *Chromohalobacter salixigens* DSM 3043^T (94.8 %), and *Chromohalobacter israelensis* ATCC 43985^T (94.8 %). The inferred phylogenetic tree showed strain EAR18^T forming a well-supported sister group with type strains of species *H. zincidurans* and *H. xinjiangensis* while all validly named species in the genus *Chromohalobacter* were allocated in a distinct clade (Fig. 1). Considering 16S rRNA gene phylogeny, the position of the genus *Chromohalobacter* could be nevertheless discussed as it appears as an ingroup in the genus *Halomonas.* Concatenated MLSA phylogenetic trees inferred including available sequences of already described species in the genera *Halomonas* and *Chromohalobacter* confirmed the unambiguous divergence of both genera (Supplementary Fig. S4, S5 and S6). Following proposed minimal standards for the use of genome data for the taxonomy of prokaryotes [55], the whole genome sequence of strain EAR18^T has a tsize of 4,648,490 bp and is formed for 142 contigs. The N50 value is 151,983, the coverage is of 41.8X, and the genomic G+C content is 64.9 mol% (Supplementary Table S1). OGRI tests were performed comparing EAR18^T genome with the genomes of *H. xinjiangensis* TRM 0175^T (JPZL01) and *H. zincidurans* B6^T (JNCK01). dDDH values were 20.4 % with *H. xinjiangensis* TRM 0175^T and 35.5 % with *H. zincidurans* B6^T (Table 3), supporting strain EAR18^T as new representative of the genus *Halomonas* [36, 55]. The same conclusion was showed by ANIb/ANIm results for strain EAR18^T and *H. xinjiangensis* TRM 0175^T (73.8 %/84.2 %) and *H. zincidurans* B6^T (86.8 %/89.4 %)

Based on phenotypic and phylogenetic data from this study is demonstrated that strain EAR18^T represents a novel species in the genus *Halomonas*, and the name *Halomonas radicis* sp. nov. is proposed.

Based on new data obtained in this study, emended descriptions of the species *H. xinjiangensis* and *H. zincidurans* are also provided.

Emended description of *Halomonas xinjiangensis* Guan et al. 2010

The properties are as given in the species description by Guan et al. (2010) with the following emendation. The polar lipids profile consists of phosphatidylglycerol and five unidentified phospholipids (the Rf values of which are documented in Suppl. Fig. S3b).

Emended description of *Halomonas zincidurans* Xu et al. 2013

The properties are as given in the species description by Xu et al. (2013) with the following emendation. The polar lipids profile consists of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, an unidentified aminophospholipid, and two unidentified phospholipids (the Rf values of which are documented in Suppl. Fig.S3c).

Description of *Halomonas radicis* sp. nov.

Halomonas radicis (ra'di.cis. L. gen. n. *radicis* of a root).

Cells are Gram negative, motile, aerobic, and non-spore forming rods frequently appearing as single short rods and 0.1 × 0.2-0.3 µm in size. It forms light orange (RAL 070 80 40), circular, and convex colonies with smooth surface and an undulate margin and a size of 1 mm diameter when grown on TSA with 2.5% NaCl (w/v), at pH 7 and 30 ºC for 2 days (optimal conditions). It grows at pH 6.0-9.0 and 15-37 ºC and tolerates 0-25 % NaCl (w/v), so it is considered a halotolerant microorganism. It grows well on MA and MacConkey, but not on cetrimide agar. Catalase positive and oxidase negative.

Starch, casein, DNA, Tween 80, chitin, cellulose, and pectin are not hydrolysed. According to API ZYM and API 20NE, strong enzymatic activity is observed for alkaline phosphatase, leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, *α*-glucosidase, pyrolidonyl arylamidase, arginine dihydrolase, and urease, weak for esterase (C4), esterase lipase (C8), and *β*-glucosidase, and negative for lipase (C14), valine arylamidase, cysteine arylamidase, trypsine, *α*-chymotrypsin, *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, N-acetyl-*β*-glucosaminadase, *α*-mannosidase, *α*-fucosidase, and *β*-glucuronidase. Negative for the hydrolysis of aesculin and gelatine and production of indole and acetoine (Voges Proskauer negative). According to API 20NE, it can reduce nitrate to nitrite, assimilate $_{D}$-glucose, $_{D}$-mannose, and potassium gluconate, but cannot ferment $_{D}$-glucose or assimilate $_{L}$-arabinose, $_{D}$-mannitol, *N*-acetyl-glucosamine, $_{D}$-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. It does not produce acid from $_{D}$-ribose, $_{L}$-arabinose, $_{D}$-mannitol, $_{D}$-sorbitol, $_{D}$-lactose, $_{D}$-trehalose, inulin, $_{D}$-raffinose, starch, and glycogen. According to Biolog GEN III system, it is positive for the oxidation of *N*-acetyl-$_{D}$-glucosamine, $_{D}$-glucose, $_{D}$-mannose, $_{D}$-fructose, $_{D}$-galactose, $_{D}$-fucose, $_{L}$-rhamnose, inosine, 1% sodium lactate, $_{D}$-sorbitol, $_{D}$-mannitol, $_{D}$-arabitol, myo-inositol, glycerol, $_{L}$-alanine, $_{L}$-arginine, $_{L}$-glutamic acid, $_{L}$-histidine, $_{L}$-pyroglutamic acid, $_{L}$-serine, $_{D}$-galacturonic acid, $_{L}$-galactonic acid-*γ*-lactone, $_{D}$-gluconic acid, $_{D}$-glucuronic acid, mucic acid, quinic acid, $_{D}$-saccharic acid, *p*-hydroxy-phenylacetic acid, methyl pyruvate, $_{L}$-lactic acid, *α*-keto-glutaric acid, $_{D}$-malic acid, $_{L}$-malic acid, bromo-succinic acid, lithium chloride, *γ*-amino-*n*-butyric acid, *α*-hydroxy-butyric acid, *β*-hydroxy-butyric acid, acetic acid, sodium formate, lincomycin, tetrazolium blue, and aztreonam but negative for dextrin, $_{D}$-maltose, $_{D}$-trehalose, $_{D}$-cellobiose, $_{β}$-gentiobiose, sucrose, turanose, stachyose, $_{D}$-raffinose, *α*-$_{D}$-lactose, $_{D}$-melibiose, *β*-methyl-$_{D}$-glucoside, $_{D}$-salicin, *N*-acetyl-*β*-$_{D}$-mannosamine, *N*-acetyl-$_{D}$-galactosamine, *N*-acetyl-neuraminic acid, 3-*O*-methyl-$_{D}$-glucose, $_{L}$-fucose, fusidic acid, $_{D}$-glucose-6-phosphate, $_{D}$-fructose-6-phosphate, $_{D}$-aspartic acid, $_{D}$-serine, gelatin, glycine-proline, $_{L}$-aspartic acid, guanidine hydrochloride, pectin, glucuronamide, $_{D}$-lactic acid methyl ester, citric acid, nalidixic acid, tween 40, *α*-keto-butyric acid, acetoacetic acid, propionic acid, butyric acid, sodium bromate, troleandomycin, rifamycin SV, minocycline, niaproof, vancomycin, tetrazolium violet, and potassium tellurite. The major fatty acids are $C_{19:0}$ cyclo *ω*8*c*, summed feature 8 ($C_{18:1}$ *ω*7*c* and/or $C_{18:1}$ *ω*6*c*), and $C_{16:0}$. The predominant respiratory quinone is Q9 but Q8 is also significant. Polar lipids profile consisted of

diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, and four unidentified phospholipids.

The type strain, EAR18^T (=CECT 9077^T=LMG 29859^T), was isolated from the root of *Arthrocnemum macrostachyum* as an endophyte. The GenBank accession number for the 16S rRNA gene sequence is KU320882. The GenBank/EMBL/DDBJ accession number for the draft genome is CAAHFN01.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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Fig. 1. Maximum-likelihood phylogenetic tree inferred from 16S rRNA gene sequences, showing the phylogenetic position of strain EAR18T relative to type strains of species within the family *Halomonadaceae*. The branches are scaled in terms of the expected number of substitutions per site. Support values obtained from 1000 replicates from maximum-likelihood (left) and maximum-parsimony (right) bootstrapping are shown above the branches if ⩾60%. Sequence accession numbers are given in parentheses.

Table 1. Differential characteristics of strains EAR18^T and the other strains of the genus *Halomonas*.

Strains (listed by 16S rRNA gene sequence similary to strain under study): 1, EAR18^T; 2, *H. zincidurans* JCM 18472^T ; 3, *H. xinjiangensis* KCTC 22608^T ; 4, *H. aestuarii* Hb3^T; 5, *H. endophytica* MC28^T; 6, *H. garicola* JJ-M1^T; 7, *H. malpeensis* YU-PRIM-29^T; 8, *H. elongata* CECT 4279^T; 9, *H. salicampi* BH103^T; 10, *H. saliphila* LCB169^T. +, positive; -, negative; ND, no data available. Data were obtained from this study under standardised conditions. Referenced results displayed were produced by following the same procedure or system to those used in this study.

Characteristics	1	2	3	4	5	6	7	8	9	10
NaCl for growth (%, w/v)										
Range	0-25	0.5-15[a]	0-20[b]	1-20[c]	0.5-6[d]	3-22.5[e]	0-20[f]	0-20[g]	0-23[h]	0-17[i]
Optimum	2.5	5[a]	10-13[b]	3-10[c]	3[d]	10[e]	0.5-5[f]	3-8[g]	14[h]	10-15[i]
pH range	6-9	5-8.5[a]	6-9[b]	5-9.5[c]	6-9[d]	5.5-9.5[e]	6-12[f]	5-10[g]	7-10.8[h]	6-10[i]
Optimum	7	7[a]	7[b]	6-6.5[c]	8.5[d]	7[e]	7-9[f]	ND	8.5[h]	8[i]
Temperature range (ºC)	15-37	4-37[a]	15-50[b]	5-45[c]	10-45[d]	20-37[e]	10-36[f]	4-45[g]	10-55[h]	10-52[i]
Optimum	30	35[a]	37[b]	25-35[c]	40[d]	30[e]	25-30[f]	ND	28[h]	30[i]
Hydrolysis of:										
Aesculin	-	-[a]	+[b]	-[c]	ND	-[e]	ND	-[g]	-[h]	+[i]
According to API 20NE, acid is produced from:										
$_D$-ribose	-	+[a]	-[a]	ND	+[d]	ND	-[f]	+[a]	+[h]	+[i]
$_L$-arabinose	-	+[a]	-[a]	+[c]	+[d]	-[e]	-[f]	+[aeg]	-h	+[i]
$_D$-sorbitol	-	+[a]	-[a]	-[c]	+[d]	-[e]	-[f]	+[aeg]	-[h]	+[i]
$_D$-trehalose	-	-[a]	+[a]	+[c]	+[d]	-[e]	-[f]	+[ae]/-[g]	+[h]	+[i]
According to GEN III system, it oxidises:										
$_D$- maltose	-	+	-	+[c]	ND	ND	ND	ND	ND	ND
$_D$-trehalose	-	+	+	+[c]	ND	ND	ND	ND	ND	ND
Sucrose	-	+	-	+[c]	ND	ND	ND	ND	ND	ND
$_D$-fructose	-	+	-	-[c]	ND	ND	ND	ND	ND	ND
$_L$-pyroglutamic acid	+	-	+	+[c]	ND	ND	ND	ND	ND	ND
$_L$-serine	+	-	+	+[c]	ND	ND	ND	ND	ND	ND
Acetoacetic acid	-	+	+	+[c]	ND	ND	ND	ND	ND	ND
Acetic acid	+	+	+	+[c]	ND	ND	ND	ND	ND	ND
Citric acid	-	+	-	+[c]	ND	ND	ND	ND	ND	ND

[a] Data from Xu *et al.* [51].
[b] Data from Guan *et al*. [50].
[c] Data from Kho *et al*. [16].
[d] Data from Chen *et al*. [19].
[e] Data from Jung *et al*. [9].
[f] Data from Kämpfer *et al*. [18].
[g] Data from Mata *et al*. [56].
[h] Data from Lee *et al*. [11].

[1] Data from Gan *et al*. [15].

Table 2. Cellular fatty acid compositions (%) of EAR18^T and closely related *Halomonas* species.

Strains: 1, strain EAR18^T; 2, *H. zincidurans* JCM 18472^T; 3, *H. xinjiangensis* KCTC 22608^T. Fatty acids amounting to < 1 % of the total fatty acids in all strains are not shown. All strains were grown on TSA with 2.5 % NaCl (w/v) for 48 h at 28 ºC. -, Not detected; tr, Trace (< 1 %). All data are obtained in this study.

Fatty acid	**1**	**2**	**3**
$C_{10:0}$	2.0	-	4.6
$C_{12:0}$	3.6	-	4.5
$C_{12:0}$ 3OH	8.1	-	7.0
iso $C_{15:0}$	-	10.4	-
anteiso $C_{15:0}$	-	37.8	-
iso $C_{16:0}$	-	9.6	-
$C_{16:0}$	19.1	9.7	7.4
iso $C_{17:0}$	tr	9.3	-
anteiso $C_{17:0}$	tr	16.9	tr
$C_{17:0}$ cyclo	9.4	-	1.2
$C_{18:1}$ ω7*c* 11-methyl	1.5	-	tr
$C_{19:0}$ cyclo ω8*c*	29.7	-	1.9
Summed Features*:			
3[a]	6.3	-	23.7
5[b]	1.1	-	-
8[c]	15.0	-	47.3

*As indicated by Montero-Calasanz *et al.* [57], summed features are groups of two or three fatty acids that are treated together for the purpose of evaluation in the MIDI System and include both peaks with discrete equivalent chain-lengths (ECLs) as well as those where the ECLs are not reported separately.

[a] Summed feature 3 is listed as $C_{16:1}$ ω7*c* and/or $C_{16:1}$ ω6*c*.

[b] Summed feature 5 is listed as anteiso-$C_{18:0}$ and/or $C_{18:2}$ ω6,9*c*.

[c] Summed feature 8 is listed as $C_{18:1}$ ω7*c* and/or $C_{18:1}$ ω6*c*.